

Clean version of all pending claims upon entry of this amendment

1. A method for constructing a DNA library *in vivo*, comprising:
 - providing a plurality of host cells;
 - providing a vector molecule having a first region and a second region;
 - providing a plurality of nucleic acid insert molecules, each of the plurality of nucleic acid insert molecules having a first common region which is homologous with said first region of the vector molecule, a second common region which is homologous with said second region of the vector molecule, and a library element encoding region disposed between said first common region and said second common region, wherein when the library element encoding region encodes a naturally occurring sequence, the first and second regions are not naturally found adjacent to the library element encoding region;
 - introducing the vector molecule into each host cell of the plurality of host cells;
 - introducing a nucleic acid insert molecule from said plurality of nucleic acid insert molecules into each host cell of said plurality of host cells, wherein a different library element encoding region is introduced into each host cell of said plurality of host cells;
 - allowing homologous recombination and gap repair between a vector molecule and a nucleic acid insert molecule to occur, and
 - generating a plurality of vector molecules from said plurality of host cells, each vector molecule comprising a different nucleic acid insert molecule,
 - thereby constructing a DNA library.
2. A method of preparing a plurality of nucleic acid insert molecules, comprising:
 - providing a plurality of nucleic acid molecules wherein each of the nucleic acid molecule includes, in order from 5' to 3', a first common sequence, a library element encoding region, and a second common sequence;
 - providing a plurality of first primers, each of said first primers having a first region which hybridizes to the first common sequence of the nucleic acid molecule and having a second region which does not hybridize to said first (and preferably second) common sequence; and
 - providing a plurality of second primers, each of said second primers having a first region which hybridizes to the second common sequence of the nucleic acid molecule and

having a second region which does not hybridize to said second (and preferably first) common sequence;

forming a reaction mixture which includes said plurality of nucleic acid molecules, said plurality of said first primers, and said plurality of said second primers, under conditions which provide a plurality of nucleic acid insert molecules having the following structure, in order from 5' to 3', a second region of said first primer/said first common region/a library element encoding region/said second common region/a second region of said second primer,

thereby preparing a plurality of nucleic acid insert molecules.

3. A method of constructing a DNA library, comprising:

providing a plurality of nucleic acid molecules wherein each of said nucleic acid molecule includes, in order from 5' to 3', a first common sequence, a library element encoding region, and a second common sequence;

providing a plurality of first primers, each of said first primers having a first region which hybridizes to the first common sequence of the nucleic acid molecule and having a second region which does not hybridize to said first (and preferably second) common sequence;

providing a plurality of second primers, each of said second primers having a first region which hybridizes to the second common sequence of the nucleic acid molecule and having a second region which does not hybridize to said second (and preferably first) common sequence;

forming a reaction mixture which includes said plurality of nucleic acid molecules, said plurality of said first primers, and said plurality of said second primers, under conditions which provide a plurality of nucleic acid insert molecules having the following structure, in order from 5' to 3', a second region of said first primer/said first common region/a library element encoding region/said second common region/a second region of said second primer;

providing a plurality of host cells;

providing a vector molecule having a first region which is homologous with said second region of said first primer, and a second region which is homologous with said second region of said second primer;

introducing said vector molecule into each host cell of the plurality of host cells;

and

introducing one or more of nucleic acid insert molecules from said plurality of nucleic acid insert molecules into each host cell of the plurality of host cells,
generating a plurality of vector molecules from said plurality of host cells, each vector molecule comprising a different nucleic acid insert molecule,
thereby providing a DNA library.

4. The method of claim 3, further comprising allowing homologous recombination and gap repair between said vector molecule and said nucleic acid insert molecule to occur.

5. The method of claim 3, wherein said first and second common sequences are the same.

6. The method of claim 3, wherein said first and second common sequences are different.

7. The method of claim 1, wherein said host cell is a yeast cell.

8. The method of claim 1, wherein said host cell is a bacterial cell.

9. The method of claim 1, wherein said at least one vector is linearized prior to being introduced into said host cell.

10. The method of claim 9, wherein said vector is linearized by cleaving between said first and second regions of said vector.

11. The method of claim 1, wherein said second common region of said nucleic acid insert molecule is produced by PCR.

12. The method of claim 1, wherein said first common region of said nucleic acid insert molecule is produced by PCR.

13. The method of claim 1, wherein said second common region of said nucleic acid insert molecule is produced by the ligation of adapters.

14. The method of claim 1, wherein said first common region of said nucleic acid insert molecule is produced by the ligation of adapters.

15. The method of claim 1, wherein said first and second common regions of said nucleic acid insert molecule are at least 30 base pairs in length.

16. The method of claim 1, wherein said first and second regions of said nucleic acid insert molecule are at least 40 base pairs in length.

17. The method of claim 1, wherein said first and second regions of said nucleic acid insert molecule are at least 50 base pairs in length.

18. The method of claim 1, wherein said library element encoding region is obtained from a cDNA library other than the one being constructed.

19. The method of claim 18, wherein said library element encoding region is obtained from a cDNA library which is plasmid based.

20. The method of claim 18, wherein said library element encoding region is obtained from a cDNA library which is phage based.

21. The method of claim 1, wherein said library element encoding region is obtained from an mRNA molecule.

22. The method of claim 21, wherein said mRNA molecule is obtained from a cancerous tissue.

23. The method of claim 1, wherein said DNA library is screened in a two-hybrid system and wherein said vector includes a transcription factor activation domain.

24. The method of claim 23, wherein said method further comprises,

introducing into each host cell of said plurality of host cells a nucleic acid molecule encoding a hybrid protein, wherein the hybrid protein comprises a transcription factor DNA-binding domain attached to a test protein;

introducing into each host cell of said plurality of host cells a detectable gene, wherein said detectable gene comprises a regulator site recognized by said DNA-binding domain and wherein said detectable gene expresses a detectable protein when said test protein interacts with a protein encoded by the DNA library;

plating each host cell of said plurality of host cells onto selective media; and

selecting for each host cell of said plurality of host cells containing a DNA encoded protein which interacts with test protein.

25. The method of claim 1, wherein said DNA library is used for screening and cloning of novel genes.

26. A method of constructing a DNA library for screening in a two-hybrid system, comprising:

providing a plurality of nucleic acid molecules, wherein each of the plurality of nucleic acid molecules includes, in order from 5' to 3', a first common sequence, a library element encoding region, and a second common sequence;

providing a plurality of first primers, each of said first primers having a first region which hybridizes to said first common sequence of said nucleic acid molecule and having a second region which does not hybridize to said first (and preferably second) common sequence;

providing a plurality of second primers, each of said second primers having a first region which hybridizes to said second common sequence of said nucleic acid molecule and having a second region which does not hybridize to said second (and preferably first) common sequence;

forming a reaction mixture which includes the plurality of nucleic acid molecules, the plurality of said first primers, and the plurality of said second primers, under conditions which provide a plurality of nucleic acid insert molecules having the following structure, in order from 5' to 3', a second region of the first primer/the first common region/a library element encoding region/the second common region/a second region of the second primer;

providing a plurality of host cells;

providing a vector having a first region which is homologous with the second region of the first primer, and a second region which is homologous with the second region of the second primer, wherein said vector further includes a transcription factor activation domain;

introducing a vector molecule into each of each host cell of said plurality of host cells;
introducing one or more of the nucleic acid insert molecules into each host cell of said plurality of host cells under conditions which allow for recombination and gap repair to occur;

introducing into each host cell of said plurality of host cells a nucleic acid molecule encoding a hybrid protein, wherein the hybrid protein includes a transcription factor DNA-binding domain attached to a test protein;

introducing into each host cell of said plurality of host cells a detectable gene, wherein said detectable gene comprises a regulator site recognized by the DNA-binding domain and wherein said detectable gene expresses a detectable protein when the test protein interacts with a protein encoded by the DNA library;

plating each host cell of said plurality of host cells onto selective media; and
selecting for each host cell of said plurality of host cells containing a DNA encoded protein which interacts with test protein.

27. A kit allowing the interchangeable use of a DNA library in more than one application, comprising:

a plurality of first PCR oligonucleotide primers, each of said first PCR primers having a first region which hybridizes to the first common sequence used in the construction of said DNA library, and a second region homologous with a first region of a vector required for a particular application;

a plurality of second PCR oligonucleotide primers, each of said second PCR primers having a first region which hybridizes to the second common sequence used in the construction of said DNA library, and a second region homologous with a second region of a vector required for a particular application; and

instructions for use.
